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Original article

Synthesis and biological activity assays of some new *N*1-(flavon-7-yl)amidrazone derivatives and related congeners

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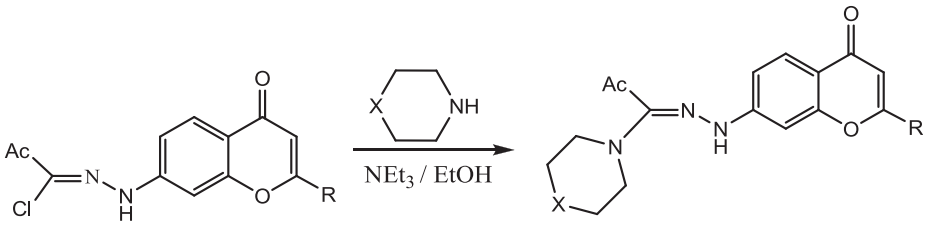
HIGHLIGHTS

- A series of new *N*1-(flavon-7-yl) amidrazones incorporating *N*-piperazines and related congeners were synthesized.
- Some of the prepared compounds exhibited high potent activity against breast cancer (MCF-7 and T47D) and Leukemic (K562) cell lines.
- The best results were obtained by compounds **5a**, **15a**, and **18b** against T47D cell line with IC_{50} values of 1.42, 1.92, and 2.92 μM, respectively.

GRAPHICAL ABSTRACT



(R = Ph / Me); (X = *N*-alkyl / *N*-Aryl)

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ABSTRACT

A series of new *N*1-(flavon-7-yl)amidrazones incorporating *N*-piperazines and related congeners were synthesized by reacting the hydrazonoyl chloride derived from 7-aminoflavone and 7-amino-2-methylchromen-4-one with the appropriate piperazine. The chemical structures of the newly prepared compounds were confirmed by elemental analyses, ^{1}H NMR, ^{13}C NMR, and ESI-HRMS spectral data. The antitumor activity of these compounds was evaluated on breast cancer (MCF-7 and T47D) and Leukemic (K562) cell lines by a cell viability assay utilizing the tetrazolium dye 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). Although with varying degrees, a significant growth inhibitory and cytotoxic effect was observed on all three cancer cell lines. Among the compounds tested compounds, **5a**, **15a**, and **18b**, were the most active against T47D cell line with IC_{50} values of 1.42, 1.92, and 2.92 μM, respectively. By using other cancer cell lines and with further characterization of their biological mechanism of action, these compounds could prove to be useful candidates as anticancer drugs.

1. Introduction

Chromones occur widely in nature, and have attracted much synthetic interest because of their reactivity and biological activity of their naturally-occurring representatives [1]. Chromones are well-known for their antioxidant activity [2], a property that stems

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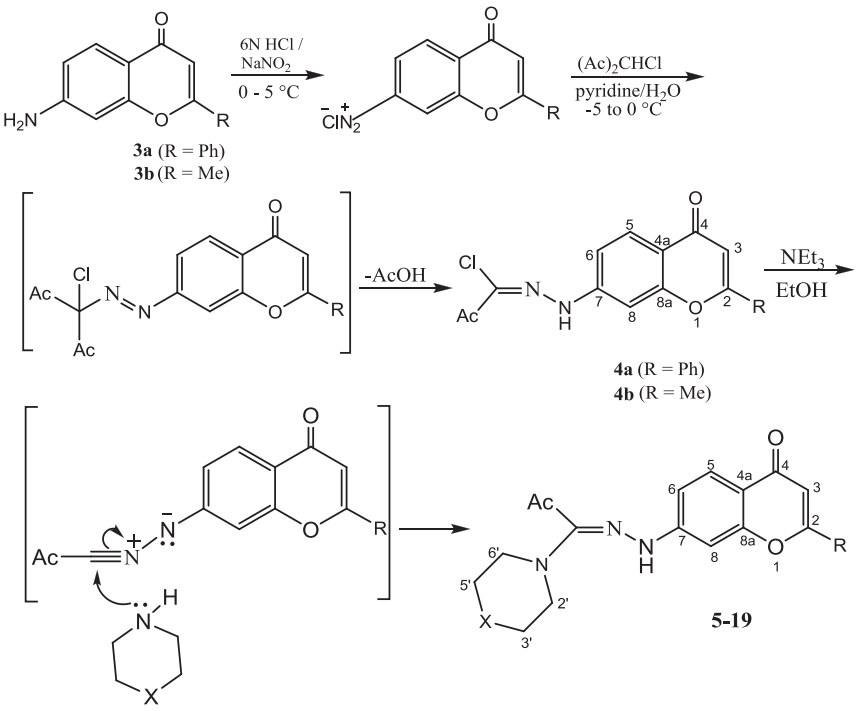


compd. No.	R	X
5a	Ph	NH
6a	Ph	NCH_3
7a	Ph	NEt
7b	CH_3	NEt
8a	Ph	NBz
8b	CH_3	NBz
9a	Ph	NCO_2Et
9b	CH_3	NCO_2Et
10b	CH_3	NCH_2CH_2OH

compd. No.	R	X
11a	Ph	CH_2
11b	CH_3	CH_2
12a	Ph	O
12b	CH_3	O
13a	Ph	S
13b	CH_3	S
14a	Ph	NPh
14b	CH_3	NPh

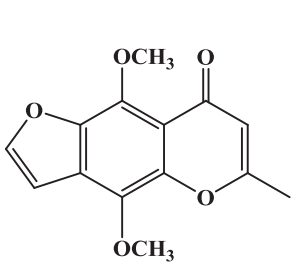
compd. No.	R	X
15a	Ph	N(*p*-C_6H_4F)
15b	CH_3	N(*p*-C_6H_4F)
16a	Ph	N(*o*-C_6H_4F)
16b	CH_3	N(*o*-C_6H_4F)
17a	Ph	N(2-pyrimidyl)
17b	CH_3	N(2-pyrimidyl)
18a	Ph	N(*p*-C_6H_4OMe)
18b	CH_3	N(*p*-C_6H_4OMe)
19a	Ph	N(*p*-C_6H_4Cl)

Scheme 1. Synthesis of compounds **4**–**19**.

from their ability to neutralize active forms of oxygen and to cut off free radical processes [3]. They also possess low mammalian toxicity and are present in large amounts in the diet of humans due to their origin in plants [4], especially in fruits and vegetables [5]. Flavonoids, a group of benzopyrone derivatives [6], have been recognized as one of the largest and most widespread class of plant constituents occurring throughout the plant kingdom [7]. A great number of biological aspects were reported for flavones [8], such as biocidal [9], immune-stimulatory [10], anti-ulcer [11], anti-estrogenic [12], antitumor [13], anti-allergic [14], anti-inflammatory [15], anti-viral [16], and anti-HIV [17] activities.

Examples include Khellin (**1**) [18] and 2,4-thiazolidenedione (**2**) [19] that are chromone derivatives used as anti-spasmodic and antidiabetic agents, respectively. These promising properties led to numerous chemical works focusing on the synthesis and the structural modifications of flavones [20].

Amino flavones are highly active molecules, wherein positions 5 and 7 are the most important and the most beneficial [21]. Flavonoids, bearing amino groups on the benzo- or pyranone ring have been reported to be potential antineoplastic agents [22]. It is now well established that such potency is mainly due to the ability of these aminoflavones to be competitive inhibitors of certain protein–tyrosine kinases with respect to ATP [23].



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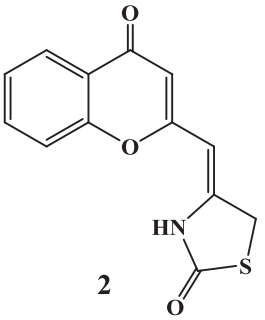

2

On the other hand, piperazine derivatives have drawn considerable attention from organic and medicinal chemists. Piperazine-based compounds have been employed as antibacterial [24], antidepressant [25], and antitumor [26] drugs, and as α-adrenoceptor antagonists [27]. Quite recently, several synthetic *N*-arylpiperazinyl amidrazones were reported to display significant antitumor activity against a number of human tumor cell lines [28]. Accordingly, we envisaged to prepare a set of amidrazones incorporating flavone moiety for evaluation of their antitumor activity.

2. Results and discussion

2.1. Chemistry

The hydrazonoyl chlorides **4a** and **4b**, required in this study, were prepared *via* direct coupling of the respective flavon-7-diazonium chloride or 2-methylchromenone-7-diazonium chloride with 3-chloropentane-2,4-dione in aqueous pyridine (Japp–Klingemann reaction) [29, 30] (Scheme 1). The latter 7-diazonium chlorides are freshly prepared by diazotization of the respective 7-amino-compounds (**3a** and **3b**), (suspended in 6 N aq. HCl).

Piperazine, *N*-substituted piperazines and related cyclic secondary amine congeners, acting as nitrogen nucleophiles, are expected to add readily onto *N*-(falvon-7-yl) or *N*-(2-methylchromenone-7-yl)nitrile imines (the reactive 1,3-dipolar species generated *in situ* from their corresponding hydrazonoyl chloride precursors **4a** and **4b** in the presence of triethylamine) to give the respective amidrazone adducts **5a**–**9a**, **11a**–**19a** and **7b**–**18b** (Scheme 1). This mode of nucleophilic addition reaction of various nucleophiles onto 1,3-dipoles is well documented [31–39] and several adducts related to **5**–**19** were obtained from the reaction of amines with hydrazonoyl chlorides.

The newly synthesized compounds **4**–**19** were characterized by elemental analyses, MS and NMR spectral data. These data, detailed in the experimental part, are consistent with the suggested structures. Thus, the mass spectra display the correct molecular ion peaks for which the measured high resolution (HRMS) data are in good agreement with the calculated values. DEPT and 2D (COSY, HMQC, HMBC) experiments showed correlations that helped in the ^{1}H- and ^{13}C-signal assignments to the different carbons and their attached, and/or neighboring hydrogens.

2.2. Biology

The antitumor activity of the synthesized compounds was characterized by conducting cell viability assay using tetrazolium dye 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). Cultures of the breast cancer cell lines MCF-7 and the Leukemic cell line K562 leukemia were treated with the target compounds first at one concentration of 50 μM and the results are shown in Table 1. In the MCF-7 screening test, ten compounds showed anti-MCF-7 activity. Those compounds were able to reduce the viability after 72 h to less than 50%. In the case of K562 cells, only six compounds illustrated anti-K562 activity. Further we determined the IC_{50} values for the potential compounds against the MCF-7 and K562 (Table 2). We also explored the activity against one additional breast cancer cell line called T47D. Interestingly, five compounds showed high potency against MCF-7, whereby they scored IC_{50} values less than 10 μM (Table 2). On the T47D scenario, more encouraging results were obtained. Here the number of compounds that scored an IC_{50} of less 10 μM increased to six and the IC_{50} values were lower than those scored in the MCF-7 case reaching in **5a** and **15a** cases an IC_{50} value of less than 2 μM (Table 2). Finally, the IC_{50} values against the K562 cell line were also encouraging, although only two compounds showed IC_{50} values less than 10 μM. Importantly, compound **11a** scored the lowest IC_{50} values against K562 although its potency against MCF-7 and T47D was not the best (Table 2).

Table 1
Percentage cell survival of MCF-7 and K562 following 72 h exposure to 50 μM of all compounds. Doxorubicin is used as a positive control.

Compound	MCF-7% survival ± SD	K562% survival ± SD
Doxorubicin	23.6 ± 1.67	11.90 ± 0.90
(**5a**)	10.28 ± 0.02	7.69 ± 0.07
(**6a**)	7.98 ± 0.24	5.18 ± 0.01
(**7a**)	9.77 ± 0.46	15.88 ± 0.02
(**7b**)	69.53 ± 0.02	77.42 ± 0.04
(**8a**)	44.32 ± 0.02	83.27 ± 6.10
(**8b**)	75.90 ± 4.45	92.92 ± 5.75
(**9a**)	14.36 ± 0.14	68.27 ± 0.05
(**9b**)	87.05 ± 5.74	84.38 ± 4.02
(**10b**)	82.64 ± 0.06	78.25 ± 0.08
(**11a**)	18.01 ± 0.01	44.58 ± 2.71
(**11b**)	65.13 ± 3.87	105.35 ± 6.71
(**12a**)	66.77 ± 6.19	19.83 ± 0.02
(**12b**)	11.80 ± 1.56	114.34 ± 3.09
(**13a**)	76.73 ± 7.93	94.56 ± 3.75
(**13b**)	75.64 ± 0.04	85.53 ± 5.21
(**14a**)	75.58 ± 0.01	64.85 ± 0.08
(**14b**)	65.84 ± 0.03	80.03 ± 0.05
(**15a**)	88.54 ± 0.05	60.86 ± 0.09
(**15b**)	43.94 ± 0.08	90.78 ± 2.11
(**16a**)	66.42 ± 0.04	72.62 ± 0.11
(**16b**)	104.56 ± 2.51	90.41 ± 5.12
(**17a**)	20.27 ± 0.01	83.90 ± 3.85
(**17b**)	42.80 ± 0.05	74.47 ± 6.24
(**18a**)	83.70 ± 0.10	42.19 ± 0.07
(**18b**)	20.56 ± 0.01	79.40 ± 3.43

2.3. Structure–activity relationship analysis and docking-based explanation

A comparison of the compounds' antitumor activity against different cell lines is effectively presented in Tables 1 and 2. The two sets of compounds, **5a**–**9a** and **11a**–**19a**, and **7b**–**18b** differ in the substitution on C-2; a phenyl group is present on C-2 in the first group (**5a**–**9a** and **11a**–**19a**), while a methyl group is connected to C-2 in the second group (**7b**–**18b**). The presence of the phenyl group at C-2 apparently does make a difference for the anti-K562 activity since all compounds in the first group (**5a**–**9a** and **11a**–**19a**) displayed moderate to excellent activity whereas none of the compounds having a methyl substituent at C-2 (**7b**–**18b**) showed any activity against the K562 cells lines. The same series of compounds were screened against breast cancer; results revealed that more compounds of the flavone series (where there is a phenyl group linked to C-2) and only few of the 2-methyl series displayed activity as shown in Table 1. In addition, the IC_{50} values for **5a**, **11a**, **12a** and **13a**, for the anti-breast cancer activity reveal the importance of the piperazine moiety and to a lesser extent the piperidine moiety. On the other hand, the situation is different for the anti-leukemic activity, where the anti-K562 activity decreases in the order **5a** < **11a** < **12a** < **13a**; this demonstrates the importance of the piperidine and piperazine moieties and to a lesser extent for thiomorpholine ring system.

Table 2
Effects of compounds that have shown potential activity on the screening assay T47D, MCF-7 and K562. Doxorubicin is used as a positive control.

Compound	IC_{50} T47D (μM) ± SD	IC_{50} MCF-7 (μM) ± SD	IC_{50} K562 (μM) ± SD
Doxorubicin	0.33 ± 0.05	0.31 ± 0.01	1.41 ± 0.31
(**5a**)	1.42 ± 0.13	5.91 ± 1.61	5.02 ± 0.78
(**6a**)	15.76 ± 1.38	22.37 ± 4.56	16.15 ± 4.17
(**7a**)	53.37 ± 4.03	56.79 ± 8.88	35 ± 1.06
(**9a**)	11.05 ± 0.61	13.56 ± 1.82	–
(**11a**)	8.79 ± 0.80	21.59 ± 5.87	2.56 ± 0.57
(**12a**)	–	–	20.18 ± 1.41
(**15a**)	1.92 ± 0.35	2.75 ± 0.73	–
(**15b**)	3.46 ± 1.00	6.33 ± 0.21	–
(**17a**)	4.31 ± 0.54	8.75 ± 1.38	–
(**17b**)	19.76 ± 2.06	57.75 ± 5.57	–
(**18a**)	–	–	14.07 ± 0.52
(**18b**)	2.92 ± 0.94	3.39 ± 0.88	–

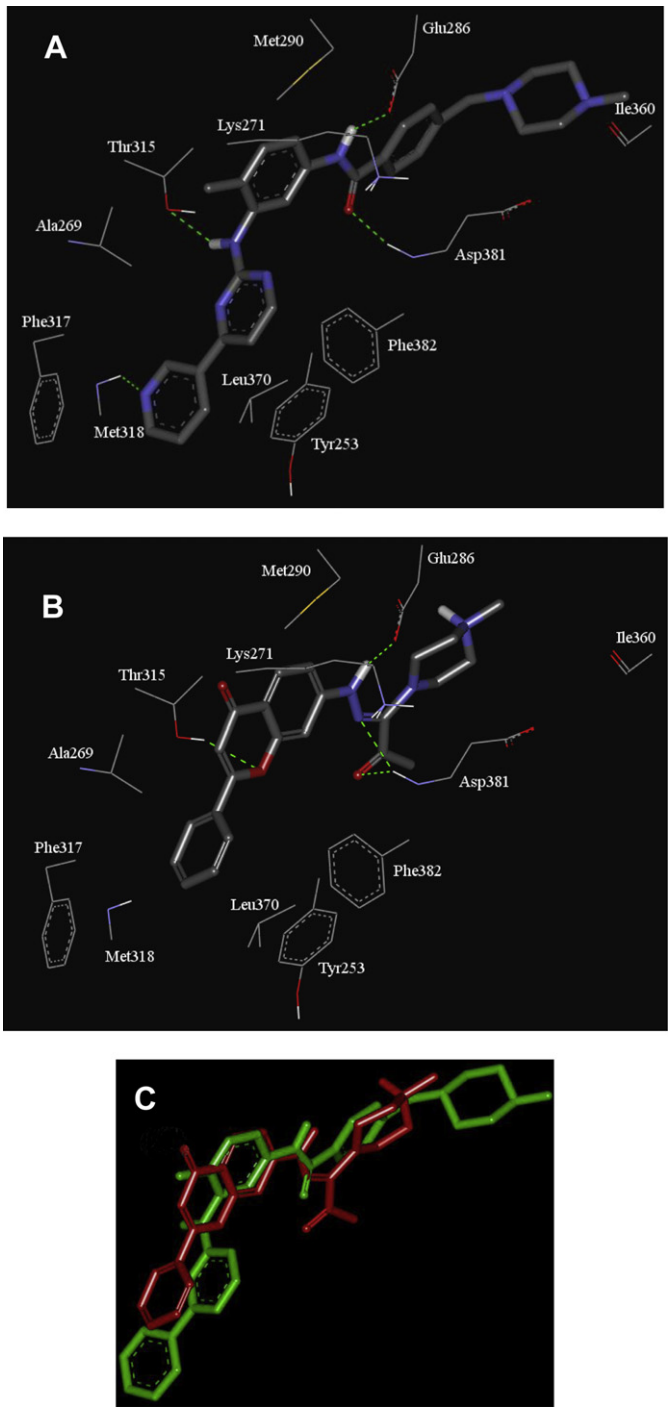

Fig. 1. (A) X-ray crystallographic structure of imatinib co-crystallized within c-abl kinase domain (PDB code: 1IEP, resolution 2.1 Å), (B) compound **6a** docked within the same binding pocket, (C) superposition of the co-crystalized structure of imatinib over the docked structure of **6a**.

Similarly, for the anti-leukemic activity, compounds **5a** and **11a**, are the most active; this finding drove us to conclude that leaving the piperazine ring unsubstituted is better for the anti-K562 activity while for breast cancer, the anti-T47D activity resembles that of the K562 activity. However, the results are more multifaceted in MCF-7, where some of the side chains resulted in negative consequences on the anti-breast cancer activity, and others have improved the activity. For example, the IC_{50} for compound **5a** has increased in many cases, a finding that emphasizes the importance of the piperazine ring. Interestingly, those compounds that have scored lower IC_{50} values than compound **5a** have longer side chains, for example, compound **8a** has an extra carbon and compound **18b** has an extra methoxy group.

The potent inhibitory effects of our new compounds against K562 and MCF-7 cancer cell lines, which over-express bcr/abl and EGFR tyrosine kinases, respectively [40,41], combined with the apparent pharmacophoric commonalities between these compounds and the anticancer agent imatinib (in particular the benzo-flavones **5a**–**9a** and **11a**–**19a**), prompted us to anticipate that their observed anticancer properties are attributable to their abilities to effectively bind and block oncogenic tyrosine kinases, particularly bcr/abl. Fig. 1 compares how imatinib binds within the ATP binding pocket of bcr/abl (PDB code: 1IEP, resolution 2.1 Å) with the way **6a** (the most active analogue, as in Table 1) docks into the binding pocket of the same protein. Clearly from the figure, positioning the pyridinyl–pyrimidine fragment of imatinib within the aromatic–hydrophobic pocket of the side chains of Phe382, Tyr253 and Phe317 (Fig. 1a), compares to fitting the phenyl substituent of **6a** into the same pocket (Fig. 1b), i.e., *via* π-stacking interactions. Similarly, hydrogen-bonding interactions connecting the amidic linker of imatinib with the carboxylic acid side chain of Glu286 and the peptidic NH of Asp381 correlate well with hydrogen-bonding interactions connecting the amidrazone fragment of **6a** with the same amino-acid residues. Similar analogy can be noticed between hydrogen-bonding interactions connecting the hydroxyl of Thr315 with the aromatic NH of imatinib (Fig. 1a) and the flavone oxygen of **6a** (Fig. 1b). Furthermore, hydrophobic stacking of the methylbenzene linker of imatinib within a narrow corridor comprised of the CH_3S- of Met290 and the $-(CH_2)_4-$ of Lys271 (Fig. 1a) compares to fitting the flavone ring of **6a** within the same corridor (Fig. 1b). Finally, the apparent electrostatic attraction connecting the piperazine ring of imatinib with the carboxylate side chain of Asp381 (Fig. 1a) corresponds to electrostatic attraction connecting the terminal piperazine nitrogen of **6a** with the same carboxylate group in the binding pocket.

3. Conclusion

In summary, we have synthesized a novel series of *N*1-(flavon-7-yl)amidrazones incorporating *N*-piperazines and related congeners through the reaction of the hydrazonoyl chloride derived from 7-aminoflavone and 7-amino-2-methylchromen-4-one with the appropriate piperazine. The prepared compounds were tested in vitro for their antitumor activity against breast cancer (MCF-7 and T47D) and Leukemic (K562) cell lines. The results revealed that a number of the synthesized compounds exerted significant anti-proliferative activity with the aforementioned cancer cell lines, particularly compounds **5a**, **15a**, and **18b**, with IC_{50} values of 1.42, 1.92, and 2.92 μM, respectively, against T47D cell line. The same compounds have IC_{50} values of 5.91, 2.75, and 3.39 μM, respectively against MCF-7. In addition, moderate antitumor activity was displayed by all of the prepared compounds against those cell lines. These findings would encourage us to do further studies and testing that prove the usefulness of the prepared compounds as candidate anticancer agents.

4. Experimental

4.1. Materials and equipments

All chemicals used were obtained from commercial sources and were used as received without further purification. 7-aminoflavone, 7-amino-2-methylchromenone, piperidine, morpholine, thiomor-

pholine, piperazine, *N*-alkylpiperazines, *N*-arylpiperazines, and *N*-acylpiperazine were purchased from Acros. Silica gel for column chromatography was received from Macherey–Nagel GmbH & Co (Germany). Melting points (uncorrected) were determined on a Stuart scientific melting point apparatus in open capillary tubes. ^{1}H and ^{13}C NMR spectra were recorded on a 300 MHz spectrometer (Bruker DPX-300) with TMS as the internal standard. Chemical shifts are expressed in δ units; ^{1}H–^{1}H, ^{1}H–F and ^{13}C–F coupling constants are given in Hertz. High resolution mass spectra (HRMS) were acquired by electrospray ionization (ESI) technique with the aid of Bruker APEX-2 instrument. The samples were dissolved in acetonitrile, diluted in spray solution (methanol/water 1:1 v/v + 0.1% formic acid) and infused using a syringe pump with a flow rate of 2 μL/min. External calibration was conducted using arginine cluster in a mass range *m*/*z* 175–871. Elemental analyses were performed on a Euro-Vector Elemental Analyzer (EA3000A).

4.2. General procedure for preparation of N-([2-substituted]-4-oxo-4H-chromen-7-yl)-2-oxo-propanehydrazonoyl chlorides (**4a**,**b**)

The title compounds were prepared by the following procedures:

Step (i). 7-aminoflavone **3a** or 7-amino-2-methylchromen-4-one **3b** (0.10 mol) was dissolved in 6 N aqueous hydrochloric acid (160 mL). To this solution was added, dropwise, a solution of sodium nitrite (7.6 g, 0.11 mol) in water (15 mL) with efficient stirring at 0–5 °C. Stirring was continued for 20–30 min, and the resulting fresh cold, flavone-7-diazonium chloride/2-methylchromenone-7-diazonium chloride solution was used immediately as such for the following coupling reaction.

Step (ii). A cold (0–5 °C) freshly prepared solution of 2-methyl- or 2-phenyl-4-oxo-4*H*-chromene-7-diazonium chloride (0.1 mol) was poured onto cold solution (−5–0 °C, ice-salt bath) of 3-chloropentane-2,4-dione (13.5 g, 0.1 mol) in pyridine/water (160 mL, 3:2 v/v) with vigorous stirring. The resulting orange-colored mixture was further stirred until a solid precipitate was formed (5–10 min). The reaction mixture was then diluted with cold water (200 mL), the solid product was collected by suction filtration, washed several times with cold water, dried and recrystallized from suitable solvent.

4.2.1. 2-oxo-N-(4-oxo-2-phenyl-4H-chromen-7-yl) propanehydrazonoyl chloride **4a**

Yield: 33.0 g, 96.9%; mp: 271–272 °C (recryst. from ethanol). ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.64 (s, 3H, C*H*$_3$), 6.79 (s, 1H, H-3), 7.20 (m, 1H, H-6), 7.39 (d, J = 1.8 Hz, 1H, H-8), 7.52–7.54 (m, 3H, H-3′ + H-4′ + H-5′), 7.90–7.93 (m, 2H, H-2′ + H-6′), 8.22 (d, J = 8.5 Hz, 1H, H-5), 8.64 (s, 1H, N–H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 26.1 (*C*H$_3$), 102.6 (C-8), 107.4 (C-3), 113.8 (C-6), 118.8 (C-4a), 126.2 (C-1′), 126.8 (C-2′/C-6′), 127.0 (C-4′), 129.6 (C-3′/C-5′), 131.7 (C-7), 132.2 (C-5), 148.0 (–C=N), 157.5 (C-8a), 162.6 (C-2), 176.8 (C-4), 188.7 (O=*C*–Me). HRMS (ESI) *m*/*z*: Calcd for $C_{18}H_{13}ClN_2O_3Na$ [M + Na]$^+$ 363.05124; found 363.05069. Anal. Calcd for $C_{18}H_{13}ClN_2O_3$ (340.76 g/mol): C, 63.44, H, 3.85, N, 8.22, found: C, 63.18, H, 3.74, N, 8.04.

4.2.2. N-(2-methyl-4-oxo-4H-chromen-7-yl)-2-oxo-propanehydrazo noyl chloride **4b**

Yield: 24.2 g, 87%; mp:274–276 °C (recryst. from acetonitrile). ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.38 (s, 3H, C*H*$_3$-2), 2.40 (s, 3H, O=C–C*H*$_3$), 6.11 (s, 1H, H-3), 7.27 (d, J = 1.8 Hz, 1H, H-8), 7.40 (dd, J = 8.7, 1.8 Hz, 1H, H-6), 8.19 (d, J = 8.7 Hz, 1H, H-5), 9.56 (s, 1H, N–H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 20.7 (*C*H$_3$-2), 26.1 (O=C–*C*H$_3$), 100.9 (C-8), 110.1 (C-3), 112.5 (C-6), 118.1 (C-4a), 127.5 (C-5), 145.4 (C-7), 147.8 (–C=N), 158.0 (C-8a), 165.5 (C-2), 177.8 (C-4), 189.1 (O=*C*–Me). HRMS (ESI) *m*/*z*: Calcd for $C_{13}H_{12}ClN_2O_3$ [M + H]$^+$ 279.05364; found 279.05310. Anal. Calcd for $C_{13}H_{11}ClN_2O_3$ (278.69 g/mol): C, 56.03, H, 3.98, N, 10.05, found: C, 56.14, H, 4.02, N, 9.88.

4.3. General procedure of amidrazones preparation (**5**–**19**)

To a cold suspension (−10–0 °C) of 1.80 mmol of **4** in 20.0 mL of ethanol was added, with stirring, a solution of appropriate secondary amine (2.0 mmol) and triethylamine (3 mL) in 5 mL of ethanol. Stirring was continued at 0–5 °C for 2–4 h, and then at ambient temperature for additional 2 h then the solution poured onto water (100 mL), the resulting crude solid product was collected by suction filtration, washed with water, dried and purified on preparative silica gel TLC plates. Using the same general procedure, the following compounds were prepared:

4.3.1. 7-[2-(2-oxo-1-(piperazin-1-yl)propylidene)hydrazinyl]-2-phenyl-4H-chromen-4-one (**5a**)

Yield: 0.26 g, 33.0%; mp: 200–202 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.76 (s, 1H, N(4′)–H), 2.46 (s, 3H, C*H*$_3$), 2.98 (m, 4H, H$_2$-2′ + H$_2$-6′), 3.00 (m, 4H, H$_2$-3′ + H$_2$-5′), 6.74 (s, 1H, H-3), 7.11 (dd, J = 8.7, 1.7 Hz, 1H, H-6), 7.33 (d, J = 1.7 Hz, 1H, H-8), 7.49–7.51 (m, 3H, H-3′ + H-5′ + H-4′), 7.88–7.91 (m, 2H, H-2′ + H-6′), 8.13 (d, J = 8.7 Hz, 1H, H-5), 9.37 (s, 1H, N–H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 26.1 (*C*H$_3$), 46.6 (C-3′/C-5′), 49.3 (C-2′/C-6′), 101.1 (C-8), 107.6 (C-3), 112.7 (C-6), 118.5 (C-4a), 126.2 (C-2″/C-6″), 127.4 (C-4″), 129.1 (C-3″/C-5″), 131.5 (C-5), 131.9 (C-1″), 145.5 (C-7), 147.3 (–C=N), 158.0 (C-8a), 163.0 (C-2), 177.8 (C-4), 195.2 (O=*C*–Me). HRMS (ESI) *m*/*z*: Calcd for $C_{22}H_{23}N_4O_3$ [M + H]$^+$ 391.17702; found 391.17647. Anal. Calcd for $C_{22}H_{22}N_4O_3$ (390.44 g/mol): C, 67.68, H, 5.68, N, 14.35, found: C, 67.44, H, 5.58, N, 14.19.

4.3.2. 7-{2-[1-(4-methylpiperazin-1-yl)-2-oxopropylidene] hydrazinyl}-2-phenyl-4H-chromen-4-one (**6a**)

Yield: 0.14 g, 17.7%; mp: 145–147 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.35 (s, 3H, C*H*$_3$), 2.47 (s, 3H, C*H*$_3$–N), 2.53 (m, 4H, H$_2$-3′ + H$_2$-5′), 3.10 (m, 4H, H$_2$-2′ + H$_2$-6′), 6.76 (s, 1H, H-3), 7.12 (dd, J = 8.7, 2.0 Hz, 1H, H-6), 7.35 (d, J = 2.0 Hz, 1H, H-8), 7.49 (m, 1H, H-4″), 7.50–7.54 (m, 2H, H-3″ + H-5″), 7.90–7.94 (m, 2H, H-2″ + H-6″), 8.15 (d, J = 8.7 Hz, 1H, H-5), 9.26 (s, 1H, N–H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 26.1 (C=O–*C*H$_3$), 46.5 (N–*C*H$_3$), 48.0 (C-2′/C-6′), 55.8 (C-3′/C-5′), 101.0 (C-8), 107.6 (C-3), 112.6 (C-6), 118.5 (C-4a), 126.3 (C-2″/C-6″), 127.4 (C-4″), 129.1 (C-3″/C-5″), 131.5 (C-5), 131.9 (C-1″), 145.5 (C-7), 147.3 (–C=N), 158.0 (C-8a), 163.1 (C-2), 177.8 (C-4), 195.1 (O=*C*–Me). HRMS (ESI) *m*/*z*: Calcd for $C_{23}H_{25}N_4O_3$ [M + H]$^+$ 405.19267; found 405.19212. Anal. Calcd for $C_{23}H_{24}N_4O_3$ (404.46 g/mol): C, 68.30, H, 5.98, N, 13.85, found: C, 68.18, H, 5.92, N, 13.76.

4.3.3. 7-{2-[1-(4-ethylpiperazin-1-yl)-2-oxopropylidene] hydrazinyl}-2-phenyl-4H-chromen-4-one (**7a**)

Yield: 0.27 g, 32.1%; mp: 165–167 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.08 (t, J = 7.2 Hz, 3H, C*H*$_3$–CH$_2$–), 2.38 (s, 3H, C=O–C*H*$_3$), 2.44 (q, J = 7.2 Hz, 2H, CH$_3$–C*H*$_2$–N), 2.52 (m, 4H, H$_2$-3′ + H$_2$-5′), 3.08 (m, 4H, H$_2$-2′ + H$_2$-6′), 6.69 (s, 1H, H-3), 7.08 (dd, J = 8.7, 2.0 Hz, 1H, H-6), 7.28 (d, J = 2.0 Hz, 1H, H-8), 7.43–7.47 (m, 3H, H-3″ + H-4″ + H-5″), 7.83–7.87 (m, 2H, H-2″ + H-6″), 8.08 (d, J = 8.7 Hz, 1H, H-5), 9.26 (s, 1H, N–H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.0 (CH$_3$–CH$_2$), 26.1 (C=O–*C*H$_3$), 48.0 (N–*C*H$_2$), 49.5 (C-2′/C-6′), 52.5 (C-3′/C-5′), 101.0 (C-8), 107.5 (C-3), 112.7 (C-6), 118.4 (C-4a), 126.2 (C-2″/C-6″), 127.3 (C-4″), 128.5 (C-3″/C-5″), 131.5 (C-5), 131.8 (C-1″), 145.5 (C-7), 147.4

(−C═N), 157.9 (C-8a), 163.0 (C-2), 177.7 (C-4), 195.1 (O═*C*−Me). HRMS (ESI) *m*/*z*: Calcd for $C_{24}H_{27}N_4O_3$ $[M + H]^+$ 419.20832; found 419.20777. Anal. Calcd for $C_{24}H_{26}N_4O_3$ (418.49 g/mol): C, 68.88, H, 6.26, N, 13.39, found: C, 68.64, H, 6.18, N, 13.26.

4.3.4. 7-{2-[1-(4-ethylpiperazin-1-yl)-2-oxopropylidene]hydrazinyl} 2-methyl-4H-chromen-4-one (**7b**)

Yield: 0.23 g, 32.0%; mp: 160–162 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.07 (t, *J* = 7.1 Hz, 3H, C*H*$_3$−CH$_2$−), 2.35 (s, 3H, C*H*$_3$-2), 2.39 (s, 3H, O═C−C*H*$_3$), 2.44 (q, 2H, *J* = 7.1 Hz, CH$_3$−C*H*$_2$−N), 2.53 (m, 4H, H$_2$-3′ + H$_2$-5′), 3.09 (m, 4H, H$_2$-2′ + H$_2$-6′), 6.10 (s, 1H, H-3), 7.06 (dd, *J* = 8.7 Hz, 2.0 Hz, 1H, H-6), 7.21 (d, *J* = 2.0 Hz, 1H, H-8), 8.08 (d, *J* = 8.7 Hz, 1H, H-5), 9.29 (s, 1H, N−H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.1 (*C*H$_3$−CH$_2$), 20.6 (*C*H$_3$-2), 26.1 (O═C−*C*H$_3$), 47.7 (N−*C*H$_2$), 49.7 (C-2′/C-6′), 52.8 (C-3′/C-5′), 100.7 (C-8), 110.5 (C-3), 112.3 (C-6), 118.3 (C-4a), 127.3 (C-5), 145.7 (C-7), 147.3 (−C═N), 158.1 (C-8a), 165.7 (C-2), 177.8 (C-4), 195.1 (O═*C*−Me). HRMS (ESI) *m*/*z*: Calcd for $C_{19}H_{25}N_4O_3$ $[M + H]^+$ 357.19267; found 357.19212. Anal. Calcd for $C_{19}H_{24}N_4O_3$ (356.42 g/mol): C, 64.03, H, 6.79, N, 15.72, found: C, 64.12, H, 6.68, N, 15.56.

4.3.5. 7-{2-[1-(4-benzylpiperazin-1-yl)-2-oxopropylidene] hydrazinyl}-2-phenyl-4H-chromen-4-one (**8a**)

Yield: 0.87 g, 90.4%; mp: 194–195 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.47 (s, 3H, C*H*$_3$), 2.56 (m, 4H, H$_2$-3′ + H$_2$-5′), 3.09 (m, 4H, H$_2$-2′ + H$_2$-6′), 3.57 (s, 2H, N−C*H*$_2$−), 6.75 (s, 1H, H-3), 7.11 (dd, *J* = 8.7, 2.0 Hz, 1H, H-6), 7.24–7.34 (m, 5H, H-2‴ + H-3‴ + H-4‴ + H-5‴ + H-6‴), 7.36 (d, *J* = 2.2 Hz, 1H, H-8), 7.49 (m, 1H, H-4″), 7.50–7.52 (m, 2H, H-3″ + H-5″), 7.90–7.93 (m, 2H, H-2′ + H-6′), 8.14 (d, *J* = 8.7 Hz, 1H, H-5), 9.28 (s, 1H, N−H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 26.2 (*C*H$_3$), 48.0 (C-2′/C-6′), 53.8 (C-3′/C-5′), 63.2 (*C*H$_2$−N), 101.0 (C-8), 107.6 (C-3), 112.7 (C-6), 118.5 (C-4a), 126.3 (C-2″/C-6″), 127.3 (C-4″), 127.4 (C-4‴), 128.4 (C-3‴/C-5‴), 129.1 (C-3″/C-5″), 129.2 (C-2‴/C-6‴), 131.5 (C-5), 131.9(C-1″), 137.9 (C-1‴), 145.6 (C-7), 147.4 (−C═N), 158.0 (C-8a), 163.1 (C-2), 177.8 (C-4), 195.2 (O═*C*−Me). HRMS (ESI) *m*/*z*: Calcd for $C_{29}H_{29}N_4O_3$ $[M + H]^+$ 481.22397; found 481.22342. Anal. Calcd for $C_{29}H_{28}N_4O_3$ (480.56 g/mol): C, 72.48, H, 5.87, N, 11.66, found: C, 72.25, H, 5.74, N, 11.48.

4.3.6. 7-{2-[1-(4-benzylpiperazin-1-yl)-2-oxopropylidene] hydrazinyl}-2-methyl-4H-chromen-4-one (**8b**)

Yield: 0.46 g, 54.8%; mp: 190–192 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.34 (s, 3H, C*H*$_3$-2), 2.43 (s, 3H, O═C−C*H*$_3$), 2.54 (m, 4H, H$_2$-3′ + H$_2$-5′), 3.07 (m, 4H, H$_2$-2′ + H$_2$-6′), 3.56 (s, 2H, N−C*H*$_2$−), 6.09 (s, 1H, H-3), 7.05 (dd, *J* = 8.7 Hz, 1.9 Hz, 1H, H-6), 7.21 (d, *J* = 1.9 Hz, 1H, H-8), 7.24–7.33 (m, 5H, H-2″ + H-3″ + H-4″ + H-5″ + H-6″), 8.08 (d, *J* = 8.7 Hz, 1H, H-5), 9.23 (s, 1H, N−H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 20.6 (*C*H$_3$-2), 26.0 (O═C−*C*H$_3$), 48.0 (C-2′/C-6′), 53.8 (C-3′/C-5′), 63.2 (N−*C*H$_2$), 100.8 (C-8), 110.5 (C-3), 112.3 (C-6), 118.0 (C-4a), 127.2 (C-5), 127.3 (C-4″), 128.4 (C-2″/C-6″), 129.1 (C-3″/C-5″), 137.9 (C-1″), 145.5 (C-7), 147.1 (−C═N), 158.2 (C-8a), 165.7 (C-2), 177.7 (C-4), 195.1 (O═*C*−Me). HRMS (ESI) *m*/*z*: Calcd for $C_{24}H_{27}N_4O_3$ $[M + H]^+$ 419.20832; found 419.20792. Anal. Calcd for $C_{24}H_{26}N_4O_3$ (418.49 g/mol): C, 68.88, H, 6.26, N, 13.39, found: C, 68.72, H, 6.18, N, 13.45.

4.3.7. Ethyl 4-[2-oxo-1-(2-(4-oxo-2-phenyl-4H-chromen-7-yl) hydrazono)propyl] piperazine-1-carboxylate (**9a**)

Yield: 0.59 g, 63.3%; mp: 165–166 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.17 (t, *J* = 7.0 Hz, 3H, C*H*$_3$−CH$_2$), 2.39 (s, 3H, O═C−C*H*$_3$), 2.91 (m, 4H, H$_2$-3′ + H$_2$-5′), 3.53 (m, 4H, H$_2$-2′ + H$_2$-6′), 4.03 (d, *J* = 7.0 Hz, 2H, MeC*H*$_2$), 6.91 (s, 1H, H-3), 7.50–7.57 (m, 5H, H-3″ + H-4″ + H-5″ + H-6 + H-8), 7.94 (d, *J* = 8.7 Hz, 1H, H-5), 8.03–8.06 (m, 2H, H-6″ + H-2″), 10.25 (s, 1H, N−H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 15.1 (*C*H$_3$CH$_2$−), 26.6 (O═C−*C*H$_3$), 44.0 (C-2′/C-6′), 47.8 (C-3′/C-5′), 61.3 (MeCH$_2$−), 101.6 (C-8), 107.4 (C-3), 113.6 (C-6), 117.7 (C-4a), 126.7 (C-2″/C-4″/C-6″), 129.6 (C-3″/C-5″), 131.8 (C-1″), 132.1 (C-5), 144.8 (C-7), 148.9 (−C═N), 155.2 (C-8a), 157.7 (O═*C*−N), 162.5 (C-2), 176.8 (C-4), 195.6 (O═*C*−Me). HRMS (ESI) *m*/*z*: Calcd for $C_{25}H_{27}N_4O_5$ $[M + H]^+$ 463.19814; found 463.19760. Anal. Calcd for $C_{25}H_{26}N_4O_5$ (462.50 g/mol): C, 64.92, H, 5.67, N, 12.11, found: C, 64.73, H, 5.54, N, 12.02.

4.3.8. Ethyl 4-{1-[2-(2-methyl-4-oxo-4H-chromen-7-yl)hydrazono]-2-oxopropyl}piperazine-1-carboxylate (**9b**)

Yield: 0.60 g, 75.1%; mp: 212–214 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.26 (t, *J* = 7.1 Hz, 3H, C*H*$_3$−CH$_2$), 2.32 (s, 3H, C*H*$_3$-2), 2.44 (s, 3H, O═C−C*H*$_3$), 3.02 (m, 4H, H$_2$-3′ + H$_2$-5′), 3.59 (m, 4H, H$_2$-2′ + H$_2$-6′), 4.15 (q, *J* = 7.1 Hz, 2H, MeC*H*$_2$), 6.06 (d, *J* = 1.0 Hz, 1H, H-3), 7.07 (dd, *J* = 8.8, 2.0 Hz, 1H, H-6), 7.21 (d, *J* = 2.0 Hz, 1H, H-8), 8.09 (d, *J* = 8.8 Hz, 1H, H-5), 9.29 (s, 1H, N−H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.7 (*C*H$_3$CH$_2$−), 20.6 (*C*H$_3$-2), 26.0 (O═C−*C*H$_3$), 47.9 (C-2′/C-6′), 49.9 (C-3′/C-5′), 61.7 (MeC*H*$_2$−), 101.0 (C-8), 110.5 (C-3), 112.3 (C-6), 118.3 (C-4a), 127.4 (C-5), 144.7 (C-7), 146.8 (−C═N), 155.5 (O═*C*−N), 158.1 (C-8a), 165.8 (C-2), 177.7 (C-4), 195.0 (O═*C*−Me). HRMS (ESI) *m*/*z*: Calcd for $C_{20}H_{25}N_4O_5$ $[M + H]^+$ 401.18249; found 401.18302. Anal. Calcd for $C_{20}H_{24}N_4O_5$ (400.43 g/mol): C, 59.99, H, 6.04, N, 13.99, found: C, 60.12, H, 5.96, N, 13.84.

4.3.9. 7-{2-[1-(4-(2-hydroxyethyl)piperazin-1-yl)-2-oxopropylidene] hydrazinyl}-2-methyl-4H-chromen-4-one (**10b**)

Yield: 0.48 g, 64.3%; mp: 147–149 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.33 (s, 3H, C*H*$_3$-2), 2.43 (s, 3H, O═C−C*H*$_3$), 2.61 (t, *J* = 5.6 Hz, 2H, −NC*H*$_2$CH$_2$OH), 2.64 (m, 4H, H$_2$-3′ + H$_2$-5′), 3.09 (m, 4H, H$_2$-2′ + H$_2$-6′), 3.63 (t, *J* = 5.6 Hz, 2H, −NCH$_2$C*H*$_2$OH), 6.09 (s, 1H, H-3), 7.05 (dd, *J* = 8.7, 2.0 Hz, 1H, H-6), 7.21 (d, *J* = 2.0 Hz, 1H, H-8), 8.08 (d, *J* = 8.7 Hz, 1H, H-5), 9.23 (s, 1H, N−H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 20.6 (*C*H$_3$-2), 26.0 (O═C−*C*H$_3$), 48.0 (C-2′/C-6′), 53.5 (C-3′/C-5′), 57.8 (−NC*H*$_2$CH$_2$OH), 59.5 (−NCH$_2$*C*H$_2$OH), 100.9 (C-8), 110.5 (C-3), 112.3 (C-6), 118.1 (C-4a), 127.3 (C-5), 145.2 (C-7), 147.0 (−C═N), 158.2 (C-8a), 165.7 (C-2), 177.7 (C-4), 195.1 (O═*C*−Me). HRMS (ESI) *m*/*z*: Calcd for $C_{19}H_{25}N_4O_4$ $[M + H]^+$ 373.18758; found 373.18680. Anal. Calcd for $C_{19}H_{24}N_4O_4$ (372.42 g/mol): C, 61.28, H, 6.50, N, 15.04, found: C, 61.06, H, 6.42, N, 14.92.

4.3.10. 7-[2-(2-oxo-1-(piperidin-1-yl)propylidene)hydrazinyl]-2-phenyl-4H-chromen-4-one (**11a**)

Yield: 0.59 g, 75.7%; mp: 172–173 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.62 (m, 6H, H$_2$-3′ + H$_2$-4′ + H$_2$-5′), 2.45 (s, 3H, C*H*$_3$), 3.00 (m, 4H, H$_2$-2′ + H$_2$-6′), 6.73 (s, 1H, H-3), 7.10 (dd, *J* = 8.7, 2.0 Hz, 1H, H-6), 7.32 (d, *J* = 2.0 Hz, 1H, H-8), 7.49–7.51 (m, 3H, H-3″ + H-4″ + H-5″), 7.88–7.91 (m, 2H, H-2″ + H-6″), 8.12 (d, *J* = 8.7 Hz, 1H, H-5), 9.28 (s, 1H, N−H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 24.0 (*C*H$_3$), 26.2 (C-4′), 26.7 (C-3′/C-5′), 49.3 (C-2′/C-6′), 100.9 (C-8), 107.6 (C-3), 112.6 (C-6), 118.3 (C-4a), 126.2 (C-2″/C-6″), 127.3 (C-4″), 129.0 (C-3″/C-5″), 131.5 (C-5), 131.9 (C-1″), 146.7 (C-7), 147.5 (−C═N), 158.0 (C-8a), 163.0 (C-2), 177.8 (C-4), 195.4 (O═*C*−Me). HRMS (ESI) *m*/*z*: Calcd for $C_{23}H_{24}N_3O_3$ $[M + H]^+$ 390.18177; found 390.18122. Anal. Calcd for $C_{23}H_{23}N_3O_3$ (389.45 g/mol): C, 70.93, H, 5.95, N, 10.79, found: C, 70.68, H, 5.86, N, 10.66.

4.3.11. 2-methyl-7-{2-[2-oxo-1-(piperidin-1-yl)propylidene] hydrazinyl}-4H-chromen-4-one (**11b**)

Yield: 0.56 g, 85.2%; mp: 206–208 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.61 (m, 6H, H$_2$-3′ + H$_2$-4′ + H$_2$-5′), 2.33 (s, 3H, C*H*$_3$-2), 2.42 (s, 3H, O═C−C*H*$_3$), 2.95 (m, 4H, H$_2$-2′ + H$_2$-6′), 6.07 (s, 1H, H-3), 7.04 (dd, *J* = 8.7, 1.9 Hz, 1H, H-6), 7.19 (d, *J* = 1.9 Hz, 1H, H-8), 8.07 (d, *J* = 8.7 Hz, 1H, H-5), 9.29 (s, 1H, N−H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 20.6 (*C*H$_3$-2), 24.4 (C-4′), 26.0 (O═C−*C*H$_3$), 26.7 (C-3′/C-5′), 49.2 (C-2′/C-6′), 100.4 (C-8), 110.4 (C-3), 112.3 (C-6), 117.9 (C-4a), 127.2

(C-5), 146.6 (C-7), 147.2 (–C═N), 158.2 (C-8a), 165.6 (C-2), 177.7 (C-4), 195.5 (O═*C*–Me). HRMS (ESI) *m*/*z*: Calcd for $C_{18}H_{22}N_3O_3$ $[M + H]^+$ 328.16612; found 328.16557. Anal. Calcd for $C_{18}H_{21}N_3O_3$ (327.38 g/mol): C, 66.04, H, 6.47, N, 12.84, found: C, 65.87, H, 6.38, N, 12.71.

4.3.12. 7-[2-(1-morpholino-2-oxopropylidene)hydrazinyl]-2-phenyl-4H-chromen-4-one (**12a**)

Yield: 0.41 g, 51.9%; mp: 209–210 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.47 (s, 3H, C*H*$_3$), 3.08 (t, *J* = 4.5 Hz, 4H, H_2-2′ + H_2-6′), 3.8 (t, *J* = 4.5 Hz, 4H, H_2-3′ + H_2-5′), 6.72 (s, 1H, H-3), 7.13 (dd, *J* = 8.7, 2.0 Hz, 1H, H-6), 7.34 (d, *J* = 2.0 Hz, 1H, H-8), 7.46–7.50 (m, 3H, H-3″ + H-4″ + H-5″), 7.87–7.90 (m, 2H, H-2″ + H-6″), 8.12 (d, *J* = 8.7 Hz, 1H, H-5), 9.41 (s, 1H, N–H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 26.1 (*C*H$_3$), 48.3 (C-2′/C-6′), 67.4 (C-3′/C-5′), 101.2 (C-8), 107.6 (C-3), 112.7 (C-6), 118.6 (C-4a), 126.2 (C-2″/C-6″), 127.4 (C-4″), 129.1 (C-3″/C-5″), 131.6 (C-5), 131.8 (C-1″), 144.7 (C-7), 147.2 (–C═N), 157.9 (C-8a), 163.1 (C-2), 177.7 (C-4), 195.1 (O═*C*–Me). HRMS (ESI) *m*/*z*: Calcd for $C_{22}H_{20}N_3O_4$ $[M - H]^-$ 390.14538; found 390.14593. Anal. Calcd for $C_{22}H_{21}N_3O_4$ (391.42 g/mol): C, 67.51, H, 5.41, N, 10.74, found: C, 67.32, H, 5.44, N, 10.63.

4.3.13. 2-methyl-7-{2-[1-morpholino-2-oxopropylidene] hydrazinyl}-4H-chromen-4-one (**12b**)

Yield: 0.48 g, 72.6%; mp: 242–244 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.35 (s, 3H, C*H*$_3$-2), 2.45 (s, 3H, O═C–C*H*$_3$), 3.07 (m, 4H, H_2-2′ + H_2-6′), 3.79 (m, 4H, H_2-3′ + H_2-5′), 6.10 (s, 1H, H-3), 7.08 (dd, *J* = 8.6, 1.8 Hz, 1H, H-6), 7.22 (d, *J* = 1.8 Hz, 1H, H-8), 8.10 (d, *J* = 8.6 Hz, 1H, H-5), 9.32 (s, 1H, N–H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 20.6 (*C*H$_3$-2), 26.0 (O═C–*C*H$_3$), 48.2 (C-2′/C-6′), 67.5 (C-3′/C-5′), 101.0 (C-8), 110.5 (C-3), 112.3 (C-6), 118.0 (C-4a), 127.4 (C-5), 144.6 (C-7), 146.9 (–C═N), 158.2 (C-8a), 165.7 (C-2), 177.6 (C-4), 195.0 (O═*C*–Me). HRMS (ESI) *m*/*z*: Calcd for $C_{17}H_{20}N_3O_4$ $[M + H]^+$ 330.14538; found 330.14483. Anal. Calcd for $C_{17}H_{19}N_3O_4$ (329.35 g/mol): C, 62.00, H, 5.81, N, 12.76, found: C, 61.84, H, 5.82, N, 12.65.

4.3.14. 7-[2-(2-oxo-1-thiomorpholinopropylidene)hydrazinyl]-2-phenyl-4H-chromen-4-one (**13a**)

Yield: 0.53 g, 65.6%; mp: 217–218 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.46 (s, 3H, C*H*$_3$), 2.75 (m, 4H, H_2-3′ + H_2-5′), 3.27 (m, 4H, H_2-2′ + H_2-6′), 6.74 (s, 1H, H-3), 7.13 (dd, *J* = 8.7, 2.0 Hz, 1H, H-6), 7.33 (d, *J* = 2.0 Hz, 1H, H-8), 7.47–7.51 (m, 3H, H-3″ + H-4″ + H-5″), 7.88–7.91 (m, 2H, H-2″ + H-6″), 8.13 (d, *J* = 8.7 Hz, 1H, H-5), 9.24 (s, 1H, N–H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 26.0 (*C*H$_3$), 28.5 (C-3′/C-5′), 50.4 (C-2′/C-6′), 101.2 (C-8), 107.6 (C-3), 112.7 (C-6), 118.6 (C-4a), 126.2 (C-2″/C-6″), 127.4 (C-4″), 129.0 (C-3″/C-5″), 131.5 (C-5), 131.8 (C-1″), 145.8 (C-7), 147.2 (–C═N), 157.9 (C-8a), 163.1 (C-2), 177.7 (C-4), 195.1 (O═*C*–Me). HRMS (ESI) *m*/*z*: Calcd for $C_{22}H_{22}N_3O_3S$ $[M + H]^+$ 408.13819; found 408.13764. Anal. Calcd for $C_{22}H_{21}N_3O_3S$ (407.49 g/mol): C, 64.85, H, 5.19, N, 10.31, found: C, 64.68, H, 5.12, N, 10.22.

4.3.15. 2-methyl-7-{2-[2-oxo-1-thiomorpholinopropylidene] hydrazinyl}-4H-chromen-4-one (**13b**)

Yield: 0.44 g, 63.4%; mp: 195–197 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.34 (s, 3H, C*H*$_3$-2), 2.43 (s, 3H, O═C–C*H*$_3$), 2.74 (m, 4H, H_2-3′ + H_2-5′), 3.26 (m, 4H, H_2-2′ + H_2-6′), 6.10 (s, 1H, H-3), 7.07 (dd, *J* = 8.5, 1.8 Hz, 1H, H-6), 7.20 (d, *J* = 1.8 Hz, 1H, H-8), 8.08 (d, *J* = 8.5 Hz, 1H, H-5), 9.17 (s, 1H, N–H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 20.6 (*C*H$_3$-2), 25.9 (O═C–*C*H$_3$), 28.5 (C-3′/C-5′), 50.4 (C-2′/C-6′), 101.0 (C-8), 110.5 (C-3), 112.3 (C-6), 118.0 (C-4a), 127.4 (C-5), 145.7 (C-7), 146.9 (–C═N), 158.2 (C-8a), 165.8 (C-2), 177.7 (C-4), 195.1 (O═*C*–Me). HRMS (ESI) *m*/*z*: Calcd for $C_{17}H_{20}N_3O_3S$ $[M + H]^+$ 346.12254; found 346.12199. Anal. Calcd for $C_{17}H_{19}N_3O_3S$ (345.42 g/mol): C, 59.11, H, 5.54, N, 12.17, found: C, 59.18, H, 5.51, N, 12.06.

4.3.16. 7-{2-[2-oxo-1-(4-phenylpiperazin-1-yl)propylidene] hydrazinyl}-2-phenyl-4H-chromen-4-one (**14a**)

Yield: 0.51 g, 54.7%; mp: 212–213 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.50 (s, 3H, C*H*$_3$), 3.18 (m, 4H, H_2-3′ + H_2-5′), 3.24 (m, 4H, H_2-2′ + H_2-6′), 6.73 (s, 1H, H-3), 6.89 (t, *J* = 7.3 Hz, 1H, H-4‴), 6.95 (d, *J* = 7.9 Hz, 2H, H-2‴ + H-6‴), 7.12 (dd, *J* = 8.7, 2.0 Hz, 1H, H-6), 7.27 (m, 2H, H-3‴ + H-5‴), 7.34 (d, *J* = 2.0 Hz, 1H, H-8), 7.49–7.51 (m, 3H, H-3″ + H-4″ + H-5″), 7.87–7.90 (m, 2H, H-2″ + H-6″), 8.13 (d, *J* = 8.7 Hz, 1H, H-5), 9.38 (s, 1H, N–H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 26.1 (*C*H$_3$), 48.2 (C-2′/C-6′), 50.1 (C-3′/C-5′), 101.2 (C-8), 107.6 (C-3), 112.7 (C-6), 116.5 (C-2‴/C-6‴), 118.6 (C-4a), 120.3 (C-4‴), 126.2 (C-2″/C-6″), 127.4 (C-4″), 129.1 (C-3″/C-5″), 129.3 (C-3‴/C-5‴), 131.5 (C-5), 131.7 (C-l″), 145.2 (C-1‴), 147.3 (C-7), 151.3 (–C═N), 158.0 (C-8a), 163.0 (C-2), 177.7 (C-4), 195.2 (O═*C*–Me). HRMS (ESI) *m*/*z*: Calcd for $[M + H]^+$ 467.20832; found 467.20777. Anal. Calcd for $C_{28}H_{26}N_4O_3$ (466.53 g/mol): C, 72.09, H, 5.62, N, 12.01, found: C, 71.93, H, 5.51, N, 11.89.

4.3.17. 2-methyl-7-{2-[2-oxo-1-(4-phenylpiperazin-1-yl) propylidene]hydrazinyl}-4H-chromen-4-one (**14b**)

Yield: 0.52 g, 64.2%; mp: 257–258 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.34 (s, 3H, C*H*$_3$-2), 2.41 (s, 3H, O═C–C*H*$_3$), 3.24 (m, 4H, H_2-3′ + H_2-5′), 3.26 (m, 4H, H_2-2′ + H_2-6′), 6.09 (s, 1H, H-3), 6.89 (t, *J* = 7.3 Hz, 1H, H-4″), 6.95 (d, *J* = 8.0 Hz, 2H, H-2″ + H-6″), 7.07 (dd, *J* = 8.7 Hz, 2.0 Hz, 1H, H-6), 7.21 (d, *J* = 2.0 Hz, 1H, H-8), 7.27 (m, 2H, H-3″ + H-5″), 8.09 (d, *J* = 8.7 Hz, 1H, H-5), 9.31 (s, 1H, N–H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 20.6 (*C*H$_3$-2), 26.0 (O═C–*C*H$_3$), 48.2 (C-2′/C-6′), 50.2 (C-3′/C-5′), 100.0 (C-8), 110.5 (C-3), 112.3 (C-6), 118.1 (C-4a), 120.3 (C-4″), 116.5 (C-2″/C-6″), 127.4 (C-5), 129.2 (C-3″/C-5″), 145.1 (C-7), 147.0 (–C═N), 151.3 (C-1″), 158.2 (C-8a), 161.3 (C-2), 177.7 (C-4), 195.1 (O═*C*–Me). HRMS (ESI) *m*/*z*: Calcd for $C_{23}H_{25}N_4O_3$ $[M + H]^+$ 405.19267; found 405.19212. Anal. Calcd for $C_{23}H_{24}N_4O_3$ (404.46 g/mol): C, 68.30, H, 5.98, N, 13.85, found: C, 68.12, H, 5.95, N, 13.68.

4.3.18. 7-(2-{1-[4-(4-fluorophenyl)piperazin-1-yl]-2-oxopropylidene} hydrazinyl)-2-phenyl-4H-chromen-4-one (**15a**)

Yield: 0.25 g, 25.3%; mp: 225–227 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.50 (s, 3H, C*H*$_3$), 3.22 (m, 4H, H_2-3′ + H_2-5′), 3.25 (m, 4H, H_2-2′ + H_2-6′), 6.75 (s, 1H, H-3), 6.88–6.93 (m, 2H, H-2‴ + H-6‴), 6.94–7.00 (m, 2H, H-3‴ + H-5‴), 7.13 (dd, *J* = 8.7, 2.0 Hz, 1H, H-6), 7.35 (d, *J* = 2.0 Hz, 1H, H-8), 7.48–7.52 (m, 3H, H-3″ + H-4″ + H-5″), 7.88–7.92 (m, 2H, H-2″ + H-6″), 8.14 (d, *J* = 8.7 Hz, 1H, H-5), 9.36 (s, 1H, N–H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 26.1 (*C*H$_3$), 48.2 (C-2′/C-6′), 51.1 (C-3′/C-5′), 101.2 (C-8), 107.6 (C-3), 112.6 (C-6), 115.4 (d, $^2J_{C-F}$ = 22.0 Hz, C-3‴/C-5‴), 118.3 (d, $^3J_{C-F}$ = 7.6 Hz, C-2‴/C-6‴), 118.6 (C-4a), 126.2 (C-2″/C-6″), 127.4 (C-4″), 129.1 (C-3″/C-5″), 131.6 (C-5), 131.9 (C-1″), 145.2 (C-7), 147.3 (–C═N), 147.9 (d, $^4J_{C-F}$ = 2.3 Hz, C-1‴), 157.5 (d, $^1J_{C-F}$ = 240 Hz, C-4‴), 158.0 (C-8a), 163.1 (C-2), 177.7 (C-4), 195.2 (O═*C*–Me). HRMS (ESI) *m*/*z*: Calcd for $C_{28}H_{26}FN_4O_3$ $[M + H]^+$ 485.19442; found 485.19535. Anal. Calcd for $C_{28}H_{25}FN_4O_3$ (484.52 g/mol): C, 69.41, H, 5.20, N, 11.56, found: C, 69.18, H, 5.23, N, 11.46.

4.3.19. 7-{2-[1-(4-(4-fluorophenyl)piperazin-1-yl) -2-oxopropylidene] hydrazinyl}-2-methyl-4H-chromen-4-one (**15b**)

Yield: 0.58 g, 68.5%; mp: 218–220 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.34 (s, 3H, C*H*$_3$-2), 2.47 (s, 3H, O═C–C*H*$_3$), 3.20 (m, 4H, H_2-3′ + H_2-5′), 3.22 (m, 4H, H_2-2′ + H_2-6′), 6.09 (s, 1H, H-3), 6.88–7.00 (m, 4H, H-2″ + H-3″ + H-5″ + H-6″), 7.07 (dd, *J* = 8.7 Hz, 1.9 Hz, 1H, H-6), 7.21 (d, *J* = 1.9 Hz, 1H, H-8), 8.09 (d, *J* = 8.7 Hz, 1H, H-5), 9.27 (s, 1H, N–H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 20.6 (CH_3-4), 26.0 (O═C–*C*H$_3$), 48.2 (C-2′/C-6′), 51.1 (C-3′/C-5′), 101.0 (C-8), 110.5 (C-3), 112.3 (C-6), 115.7 (d, $^2J_{C-F}$ = 22.5 Hz, C-3″/C-5″), 118.1 (C-4a), 118.3 (d, $^3J_{C-F}$ = 7.5 Hz, C-2″/C-6″), 127.4 (C-5), 145.0 (C-7), 147.0

(−C═N), 147.7 (C-1″), 157.0 (d, $^{1}J_{C-F}$ = 240 Hz, C-4″), 158.2 (C-8a), 165.7 (C-2), 177.6 (C-4), 195.2 (O═*C*−Me). HRMS (ESI) *m*/*z*: Calcd for $C_{23}H_{24}FN_4O_5$ $[M + H]^+$ 423.18324; found 423.18270. Anal. Calcd for $C_{23}H_{23}FN_4O_3$ (422.45 g/mol): C, 65.39, H, 5.49, N, 13.26, found: C, 65.13, H, 5.41, N, 13.08.

*4.3.20. 7-(2-{1-[4-(2-fluorophenyl)piperazin-1-yl]-2-oxopropylidene} hydrazinyl)-2-phenyl-4H-chromen-4-one (**16a**)*

Yield: 0.28 g, 29.1%; mp: 225–227 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.50 (s, 3H, C*H*$_3$), 3.22 (m, 4H, H_2-3′ + H_2-5′), 3.23 (m, 4H, H_2-2′ + H_2-6′), 6.76 (s, 1H, H-3), 6.96–7.08 (m, 4H, H-3‴ + H-4‴ + H-5‴ + H-6‴), 7.14 (dd, *J* = 8.7, 2.0 Hz, 1H, H-6), 7.37 (d, *J* = 2.0 Hz, 1H, H-8), 7.50–7.53 (m, 3H, H-3″ + H-4″ + H-5″), 7.90–7.93 (m, 2H, H-2″ + H-6″), 8.15 (d, *J* = 8.7 Hz, 1H, H-5), 9.36 (s, 1H, N–H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 26.1 (*C*H$_3$), 48.3 (C-2′/C-6′), 51.4 (C-3′/C-5′), 101.2 (C-8), 107.6 (C-3), 112.7 (C-6), 116.4 (d, $^{2}J_{C-F}$ = 20.6 Hz, C-3‴), 118.6 (C-4a), 119.2 (d, $^{4}J_{C-F}$ = 2.9 Hz, C-5‴), 123.0 (d, $^{3}J_{C-F}$ = 7.9 Hz, C-4‴), 124.5 (d, $^{3}J_{C-F}$ = 3.5 Hz, C-6‴), 126.3 (C-2″/C-6″), 127.4 (C-4″), 129.1 (C-3″/C-5″), 131.6 (C-5), 131.9 (C-1″), 139.5 (d, $^{2}J_{C-F}$ = 8.6 Hz, C-1‴), 145.3 (C-7), 147.3 (−C═N), 155.5 (d, $^{1}J_{C-F}$ = 244.6 Hz, C-2‴), 158.0 (C-8a), 163.1 (C-2), 177.8 (C-4), 195.1 (O═*C*−Me). HRMS (ESI) *m*/*z*: Calcd for $C_{28}H_{26}FN_4O_3$ $[M + H]^+$ 485.19442; found 485.19435. Anal. Calcd for $C_{28}H_{25}FN_4O_3$ (484.52 g/mol): C, 69.41, H, 5.20, N, 11.56, found: C, 69.15, H, 5.14, N, 11.42.

*4.3.21. 7-{2-[1-(4-(2-fluorophenyl)piperazin-1-yl)-2-oxopropylidene] hydrazinyl}-2-methyl-4H-chromen-4-one (**16b**)*

Yield: 0.56 g, 65.9%; mp: 195–197 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.34 (s, 3H, C*H*$_3$-2), 2.47 (s, 3H, O═C−C*H*$_3$), 3.20 (m, 4H, H_2-3′ + H_2-5′), 3.23 (m, 4H, H_2-2′ + H_2-6′), 6.09 (s, 1H, H-3), 6.96–7.07 (m, 5H, H-6 + H-3″ + H-4″ + H-5″ + H-6″), 7.20 (m, 1H, H-8), 8.08 (d, *J* = 8.0 Hz, 1H, H-5), 9.31 (s, 1H, N–H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 20.6 (*C*H$_3$-2), 26.0 (O═C−*C*H$_3$), 48.2 (C-2′/C-6′), 51.3 (C-3′/C-5′), 101.0 (C-8), 110.5 (C-3), 112.3 (C-6), 118.1 (C-4a), 116.2 (d, $^{2}J_{C-F}$ = 21.0 Hz, C-3″), 119.2 (d, $^{4}J_{C-F}$ = 2.8 Hz, C-5″), 122.9 (d, $^{3}J_{C-F}$ = 8.3 Hz, C-4″), 124.6 (d, $^{3}J_{C-F}$ = 3.4 Hz, C-6″), 127.3 (C-5), 140.0 (d, $^{2}J_{C-F}$ = 9.0 Hz, C-1″), 145.1 (C-7), 147.0 (−C═N), 158.2 (C-8a), 155.8 (d, $^{1}J_{C-F}$ = 245.0 Hz, C-2″), 165.8 (C-2), 177.7 (C-4), 195.1 (O═*C*−Me). HRMS (ESI) *m*/*z*: Calcd for $C_{23}H_{22}FN_4O_3$ $[M - H]^-$ 421.16759; found 421.16705. Anal. Calcd for $C_{23}H_{23}FN_4O_3$ (422.45 g/mol): C, 65.39, H, 5.49, N, 13.26, found: C, 65.23, H, 5.46, N, 13.12.

*4.3.22. 7-{2-[2-oxo-1-(4-(pyrimidin-2-yl)piperazin-1-yl)propylidene] hydrazinyl}-2-phenyl-4H-chromen-4-one (**17a**)*

Yield: 0.64 g, 68.2%; mp: 202–203 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.48 (s, 3H, C*H*$_3$), 3.13 (m, 4H, H_2-3′ + H_2-5′), 3.94 (m, 4H, H_2-2′ + H_2-6′), 6.51 (t, *J* = 4.7 Hz, 1H, H-5‴), 6.76 (s, 1H, H-3), 7.15 (d, *J* = 8.6 Hz, 1H, H-6), 7.37 (s, 1H, H-8), 7.50–7.52 (m, 3H, H-3″ + H-4″ + H-5″), 7.90–7.92 (m, 2H, H-2″ + H-6″), 8.15 (d, *J* = 8.6 Hz, 1H, H-5), 8.32 (d, *J* = 4.7 Hz, 2H, H-4‴ + H-6‴), 9.45 (s, 1H, N–H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 26.7 (*C*H$_3$), 43.9 (C-2′/C-6′), 47.7 (C-3′ + C-5′), 101.6 (C-8), 107.3 (C-3), 110.6 (C-5‴), 113.7 (C-6), 117.7 (C-4a), 126.7 (C-2″/C-4″/C-6″), 129.7 (C-3″/C-5″), 131.8 (C-1″), 132.1 (C-5), 145.1 (C-7), 149.0 (−C═N), 157.7 (C-8a), 158.5 (C-4‴/C-6‴), 161.8 (C-2‴), 162.5 (C-2), 176.8 (C-4), 195.7 (O═*C*−Me). HRMS (ESI) *m*/*z*: Calcd for $C_{26}H_{25}N_6O_3$ $[M + H]^+$ 469.19881; found 469.19827. Anal. Calcd for $C_{26}H_{24}N_6O_3$ (468.51 g/mol): C, 66.65, H, 5.16, N, 17.94, found: C, 66.43, H, 5.06, N, 17.72.

*4.3.23. 2-methyl-7-(2-(2-oxo-1-(4-(pyrimidin-2-yl)piperazin-1-yl) propylidene)hydrazinyl)-4H-chromen-4-one (**17b**)*

Yield: 0.58 g, 71.2%; mp: 232–235 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.34 (s, 3H, C*H*$_3$-2), 2.44 (s, 3H, O═C−C*H*$_3$), 3.11 (m, 4H, H_2-3′ + H_2-5′), 3.92 (m, 4H, H_2-2′ + H_2-6′), 6.08 (s, 1H, H-3), 6.50 (t, *J* = 4.7 Hz, 1H, H-5″), 7.09 (dd, *J* = 8.7, 2.0 Hz, 1H, H-6), 7.23 (d, *J* = 2.0 Hz, 1H, H-8), 8.09 (d, *J* = 8.7 Hz, 1H, H-5), 8.32 (d, *J* = 4.7 Hz, 2H, H-4″ + H-6″), 9.39 (s, 1H, N–H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 20.6 (*C*H$_3$-2), 26.0 (O═C−*C*H$_3$), 44.3 (C-2′/C-6′), 48.0 (C-3′/C-5′), 101.0 (C-8), 110.4 (C-5″), 110.5 (C-3), 112.3 (C-6), 118.2 (C-4a), 127.4 (C-5), 145.0 (C-7), 146.9 (−C═N), 157.8 (C-4″/C-6″), 158.2 (C-8a), 161.6 (C-2″), 165.7 (C-2), 177.6 (C-4), 195.2 (O═*C*-Me). HRMS (ESI) *m*/*z*: Calcd for $C_{21}H_{23}N_6O_3$ $[M + H]^+$ 407.18316; found 407.18276. Anal. Calcd for $C_{21}H_{22}N_6O_3$ (406.44 g/mol): C, 62.06, H, 5.46, N, 20.68, found: C, 61.88, H, 5.40, N, 20.54.

*4.3.24. 7-(2-{1-[4-(4-methoxyphenyl)piperazin-1-yl]-2-oxopropylidene}hydrazinyl)-2-phenyl-4H-chromen-4-one (**18a**)*

Yield: 0.31 g, 31.6%; mp: 207–208 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.50 (s, 3H, C*H*$_3$), 3.18 (m, 4H, H_2-3′ + H_2-5′), 3.24 (m, 4H, H_2-2′ + H_2-6′), 3.76 (s, 3H, O*CH*$_3$), 6.75 (s, 1H, H-3), 6.85 (m, 2H, H-2‴ + H-6‴), 6.93 (m, 2H, H-3‴ + H-5‴), 7.12 (dd, *J* = 8.7, 2.0 Hz, 1H, H-6), 7.35 (d, *J* = 2.0 Hz, 1H, H-8), 7.48–7.52 (m, 3H, H-3″ + H-4″ + H-5″), 7.89–7.92 (m, 2H, H-2″ + H-6″), 8.14 (d, *J* = 8.7 Hz, 1H, H-5), 9.36 (s, 1H, N–H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 26.8 (*C*H$_3$), 48.0 (C-2′/C-6′), 50.1 (C-3′/C-5′), 55.7 (O*C*H$_3$), 101.5 (C-8), 107.4 (C-3), 113.6 (C-6), 114.8 (C-3‴/C-5‴), 117.6 (C-4a), 118.1 (C-2‴/C-6‴), 126.7 (C-2″/C-6″), 128.8 (C-4″), 129.6 (C-3″/C-5″), 131.1 (C-1″), 132.1 (C-5), 145.3 (C-1‴), 146.2 (C-7), 149.0 (C-4‴), 153.4 (−C═N), 157.7 (C-8a), 162.5 (C-2), 176.8 (C-4), 195.6 (O═*C*−Me). HRMS (ESI) *m*/*z*: Calcd for $C_{29}H_{29}N_4O_4$ $[M + H]^+$ 497.21888; found 497.21833. Anal. Calcd for $C_{29}H_{28}N_4O_4$ (496.56 g/mol): C, 70.15, H, 5.68, N, 11.28, found: C, 69.94, H, 5.56, N, 11.21.

*4.3.25. 7-(2-(1-(4-(4-methoxyphenyl)piperazin-1-yl)-2-oxopropylidene)hydrazinyl)-2-methyl-4H-chromen-4-one (**18b**)*

Yield: 0.76 g, 87.1%; mp: 254–256 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.34 (s, 3H, C*H*$_3$-2), 2.47 (s, 3H, O═C−C*H*$_3$), 3.17 (m, 4H, H_2-3′ + H_2-5′), 3.22 (m, 4H, H_2-2′ + H_2-6′), 3.76 (s, 3H, O−C*H*$_3$), 6.08 (s, 1H, H-3), 6.84 (d, *J* = 9.0 Hz, H-2″ + H-6″), 6.92 (d, *J* = 9.0 Hz, H-3″ + H-5″), 7.06 (dd, *J* = 8.7 Hz, 1.9 Hz, 1H, H-6), 7.21 (d, *J* = 1.9 Hz, 1H, H-8), 8.08 (d, *J* = 8.7 Hz, 1H, H-5), 9.30 (s, 1H, N–H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 20.5 (CH_3-2), 26.0 (O═C−*C*H$_3$), 48.3 (C-2′/C-6′), 51.6 (C-3′/C-5′), 55.6 (O*C*H$_3$), 100.9 (C-8), 110.4 (C-3), 112.3 (C-6), 114.5 (C-3″/C-5″), 118.2 (C-4a), 118.6 (C-2″/C-6″), 127.3 (C-5), 154.2 (C-4″), 145.7 (C-1″), 145.2 (C-7), 147.0 (−C═N), 158.3 (C-8a), 177.6 (C-4), 177.6 (C-4), 195.1 (O═*C*−Me). HRMS (ESI) *m*/*z*: Calcd for $C_{24}H_{27}N_4O_4$ $[M + H]^+$ 435.19541; found 435.20068. Anal. Calcd for $C_{24}H_{26}N_4O_4$ (434.49 g/mol): C, 66.34, H, 6.03, N, 12.89, found: C, 66.15, H, 5.95, N, 12.82.

*4.3.26. 7-(2-{1-[4-(4-chlorophenyl)piperazin-1-yl]-2-oxopropylidene} hydrazinyl)-2-phenyl-4H-chromen-4-one (**19a**)*

Yield: 0.29 g, 28.8%; mp: 204–205 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.41 (s, 3H, C*H*$_3$), 3.13 (m, 4H, H_2-3′ + H_2-5′), 3.27 (m, 4H, H_2-2′ + H_2-6′), 6.90 (s, 1H, H-3), 6.97 (d, *J* = 9.0 Hz, 2H, H-2‴ + H-6‴), 7.23 (d, *J* = 9.0, 2H, H-3‴ + H-5‴), 7.51 (dd, *J* = 8.7, 2.0 Hz, 1H, H-6), 7.53–7.59 (m, 4H, H-3″ + H-4″ + H-5″ + H-8), 7.94 (d, *J* = 8.7 Hz, 1H, H-5), 8.05 (m, 2H, H-2″ + H-6″), 10.2 (s, 1H, N–H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 26.7 (*C*H$_3$), 47.7 (C-2′/C-6′), 48.6 (C-3′/C-5′), 101.6 (C-8), 107.4 (C-3), 113.6 (C-6), 117.5 (C-2‴/C-6‴), 117.7 (C-4a), 122.8 (C-3‴/C-5‴), 126.7 (C-2″/C-6″), 129.2 (C-4″), 129.7 (C-3″/C-5″), 131.9 (C-1″), 132.1 (C-5), 141.4 (C-4‴), 145.1 (C-1‴), 149.0 (C-7), 150.5 (−C═N), 157.7 (C-8a), 162.6 (C-2), 176.8 (C-4), 195.6 (O═*C*−Me). HRMS (ESI) *m*/*z*: Calcd for $C_{28}H_{24}ClN_4O_3$ $[M - H]^-$ 499.15369; found 499.15424. Anal. Calcd for $C_{28}H_{25}ClN_4O_3$ (500.98 g/mol): C, 67.13, H, 5.03, N, 11.18, found: C, 67.04, H, 5.12, N, 11.06.

4.4. Cell lines and cell culture

The K562 leukemia cell line was obtained from Dr. Mona Hassona (Faculty of Science, The University of Jordan) and was cultured

in RPMI while the T47D and MCF-7 breast cancer cells were obtained from American Type culture collections (ATCC) and were cultured in DMEM/F12. All media were supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Gibco Invitrogen), 1% of 2 mM L-glutamine (Lonza), 50 IU/mL penicillin (Lonza), and 50 μg/mL streptomycin (Lonza) and cells were maintained at 37 °C, 5% CO_2 humidified incubator.

4.5. Cell proliferation assay

MCF-7, T47D and K562 cells were seeded at a density of 1×10^4, 1×10^4 and 4×10^4 cells per well in 96-well plates in appropriate medium, respectively. For anti-MCF-7 and anti-K562 screening, the cells were treated with 50 μM concentrations of the tested compounds. For the IC_{50} determination the cells were treated with increasing concentrations of the tested compound (1.56–100 μM). In all assays, the drugs were dissolved in DMSO immediately before the addition to cell cultures and equal amounts of the solvent were added to control cells. Cell viability was assessed, after 3 days of treatment, with tetrazolium dye 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), obtained from Sigma (Dorset, UK). IC_{50} concentrations were obtained from the dose–response curves using GraphPad Prism Software 5 (San Diego, California, USA, www.graphpad.com).

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